

INVESTIGATION OF THE PHYSICAL PROPERTIES OF CHOLESTERYL
ESTER LIQUID CRYSTAL AND THE INTERACTION WITH CELLS

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A thesis submitted in
fulfilment of the requirement for the award of the
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*For my dearest mother, father, husband and family for their encouragement and
blessing...*

To my beloved friend and their support and caring.....

-Never tired and give up to gain knowledge and life is a journey of learning-



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ABSTRACT

Cholesteryl ester liquid crystals (CELC) were demonstrated with application in biosensing and microtissue regeneration. The affinity of the cells to this liquid crystal is unclear and required further investigation. This study focused on characterising the physical properties of CELC and interaction of human keratinocytes with CELC. The physical properties of CELC were characterised by a custom built contact angle measurement system and bubble pressure measurement apparatus. Other methods such as pendant drop were applied to determine the critical surface tension of the CELC. Then, the characterization of the CELC was continued by using Differential Scanning Calorimeter (DSC), X-ray Diffraction (XRD), Polarising Microscopy (POM) and Fourier Transform Infrared Spectroscopy (FTIR). Nonetheless, the morphology of cells interaction with CELC after it reached confluency was studied using Field Emission Scanning Electron Microscopy (FESEM) and non-contact mode of Atomic Force Microscopy (AFM). The results showed that the critical surface tension of the liquid crystal using contact angle was 37.5 mN/m and the surface tension measured using pendant drop method was found to be 23.6 mN/m. Both results indicate that the surface of the liquid crystal was moderately hydrophobic. From the DSC, CELC was found stable at room and incubator temperature. From XRD results, the compound of CELC interacts in cell culture media self-assembles into lyotropic layer. POM and FTIR analysis showed CELC after immersion in media displayed lyotropic smectic phases. The AFM and FESEM images indicated good adhesion of cells on the CELC. This research thus showed that the hydrophilic layers of lyotropic phase of cholesteryl ester liquid crystal were demonstrated with biophysical properties that support the adhesion of cells.

ABSTRAK

Kristal cecair kolesterlat ester (CELC) telah menunjukkan aplikasi dalam biosensor dan pertumbuhan semula tisu mikro. Pertalian sel-sel terhadap kristal cecair ini adalah tidak jelas dan memerlukan kajian lanjut. Kajian ini memberi tumpuan kepada pencirian sifat-sifat fizikal CELC dan interaksi CELC dengan keratinosit manusia. Sifat fizikal CELC dicirikan dengan menggunakan sistem pengukuran dan kaedah pengukuran tekanan gelembung sudut yang telah direka. Kaedah-kaedah lain seperti '*pendant drop*' telah digunakan untuk menentukan ketegangan permukaan kritikal CELC itu. Pencirian CELC diteruskan dengan menggunakan mesin Kalorimeter imbasan perbezaan (DSC), difraksi sinar-X (XRD), mikroskop optis berkutub (POM) dan perubahan fourier infra-merah (FTIR). Morfologi sel selepas interaksi dengan CELC dikaji menggunakan mikroskop elektron pengimbas pancaran medan (FESEM) dan mod tanpa sentuh mikroskop daya atomik (AFM). Keputusan menunjukkan ketegangan permukaan kritikal kristal cecair menggunakan sudut sentuh adalah 37.5 mN/m dan ketegangan permukaan dengan menggunakan kaedah '*pendant drop*' ialah 23.6 mN/m. Kedua-dua keputusan menunjukkan bahawa permukaan kristal cecair adalah sederhana hidrofobik. Daripada keputusan DSC, CELC didapati stabil pada suhu bilik dan inkubator. Keputusan XRD menunjukkan, apabila CELC berinteraksi dalam sel media, ia bertukar sendiri ke lapisan lyotropic. Analisis POM dan FTIR menunjukkan CELC selepas rendaman dalam media, memaparkan fasa smectic '*lyotropic*'. Gambar-gambar dari AFM dan FESEM menunjukkan sel melekat dengan baik pada CELC tersebut. Kajian ini sekali gus telah membuktikan bahawa lapisan hidrofilik fasa *lyotropic* cecair kolesterlat ester kristal mempunyai ciri-ciri biofizikal yang menyokong lekatan sel.

CONTENTS

TITLE	i
DECLARATION	ii
DEDICATION	iii
ACKNOWLEDGEMENT	iv
LIST OF ASSOCIATED PUBLICATION	v
LIST OF AWARD	vi
ABSTRACT	vii
ABSTRAK	viii
TABLE OF CONTENTS	ix
LIST OF TABLES	xiv
LIST OF FIGURES	xv
LIST OF SYMBOLS	xx
LIST OF ABBREVIATIONS	xxii

CHAPTER 1	PROJECT OVERVIEW	
1.1	Project background	1
1.2	Problem statement	2
1.3	Objective	3
1.4	Scope of project	3
1.5	Thesis organisation	4
1.6	Thesis contribution	4

CHAPTER 2 LITERATURE REVIEW

2.1	Introduction to liquid crystal	6
2.2	Types of liquid crystal	7
2.2.1	Thermotropic liquid crystal	7
2.2.1.1	Nematic phases	8
2.2.1.2	Cholesteric phases	8
2.2.1.3	Smectic phases	10
2.2.2	Lyotropic liquid crystal	10
2.3	Application of liquid crystal in biosensing	12
2.4	Human keratinocytes (HaCaTs)	14
2.5	Cellular adhesion	15
2.6	Surface tension measuring technique	16
2.6.1	Contact angle measurement	17
2.6.2	Bubble pressure	19
2.6.3	Pendant drop	20
2.6.4	Surface tension of liquid crystal	22
2.7	Spectrophotometer	23
2.8	Differential scanning calorimeter (DSC)	23
2.9	X-ray diffractometer (XRD)	24
2.10	Fourier transform infrared spectroscopy (FTIR)	26
2.11	Microscopy	27
2.11.1	Phase contrast microscope	28
2.11.2	Atomic force microscopy (AFM)	29
2.11.3	Field emission scanning electron microscope	31

CHAPTER 3 METHODOLOGY

3.1	Introduction	34
3.2	Development of a contact angle measurement system	36
3.2.1	Experiment for validating contact angle measurement system	44

3.3	Characterising the physical properties of cholesteryl ester liquid crystal (CELC)	45
3.3.1	Preparation of cholesteryl ester liquid crystal (CELC)	45
3.3.2	Measuring surface tension of CELC	46
3.3.2.1	Contact angle method	46
3.3.2.2	Bubble pressure method	47
3.3.2.3	Pendant drop method	49
3.3.3	Thermal stability analysis	50
3.3.3.1	Preparation of CELC for differential scanning calorimeter (DSC)	50
3.3.3.2	Preparation of CELC for optical emission spectroscopy (OES)	51
3.3.4	Preparation of CELC for polarising microscopy	52
3.3.5	Liquid crystal sample preparation for x-ray diffraction (XRD)	53
3.3.6	Liquid crystal sample preparation and fourier transform infrared analysis	54
3.4	Studying the cells interaction with CELC	55
3.4.1	Cell culture	55
3.4.2	Cell culture on the liquid crystal and glass substrate	56
3.4.3	AFM of cells on the liquid crystal and glass substrate	57
3.4.4	FESEM of cells on the liquid crystal and glass substrate	58

CHAPTER 4 RESULT AND DISCUSSION

4.1	Introduction	60
4.2	The contact angle measurement system	60

4.2.1	Calibration results of contact angle measurement system	63
4.3	Physical properties of cholesteryl ester liquid crystal (CELC)	66
4.3.1	Cholesteryl ester liquid crystal (CELC)	66
4.3.2	Surface of cholesteryl ester liquid crystal	66
4.3.2.1	Contact angle	66
4.3.2.2	Bubble pressure	68
4.3.2.3	Pendant drop	70
4.3.3	Thermal stability of cholesteryl ester liquid crystal	72
4.3.4	Polarising optical microscopy (POM)	74
4.3.5	Crystallinity study of cholesteryl ester liquid crystal	76
4.3.6	Chemistry elements in cholesteryl ester liquid crystal	77
4.4	Cells interaction with cholesteryl ester liquid crystal	79
4.4.1	Morphological effect and adhesion characteristic of cells to celc	79
CHAPTER5	CONCLUSION AND FUTURE WORKS	
5.1	Conclusion	89
5.2	Future works	90
REFERENCES		92
APPENDIX A	Architecture of the Arduino UNO microcontroller board	
APPENDIX B	Specification of L293D motor driver	

APPENDIX C Arduino coding

APPENDIX D Matlab coding

APPENDIX E The data of contact angle method

APPENDIX F The data of bubble pressure method



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LIST OF TABLES

TABLE NO	TITLE	PAGE
2.1	Application of the liquid crystal as biosensor	13
2.2	Probe liquids and their interfacial tension	18
2.3	Surface tension values for liquid crystal	22
4.1	Interfacial tension value for CELC	71
4.2	Percentage of transmission calculated from Beer's law	73
4.3	Differences of AFM and FE-SEM for the application in cells imaging	88



PTTA UTHM
PERPUSTAKAAN TUNKU TUN AMINAH

LIST OF FIGURES

FIGURE NO	TITLE	PAGE
2.1	Basic structure of a liquid crystal molecule	7
2.2	A graphical illustration of cholesteric phase	8
2.3	Types of cholesteric derivatives	9
2.4	Standard thermotropic liquid crystal phases	10
2.5	Typical phases of lyotropic liquid crystal.	11
2.6	Layer of skin at epidermis consists of keratinocytes	14
2.7	A schematic diagram of cellular adhesion	15
2.8	Contact angle measurement of a droplet of liquid	17
2.9	Apparatus for surface tension measurement using bubble pressure	19
2.10	A pendant drop system from AST Products, INC	20
2.11	The pendant drop geometry	22
2.12	Light transmitted or reflected through a sample	23
2.13	Schematic DSC heating curve of a semi-crystalline polymer	24
2.14	PANalytical X'Pert Powder X-Ray Diffraction	25
2.15	Setup for a scattering experiment utilizing X-ray diffraction	25

2.16	Fourier transform infrared spectroscopy (Perkin Elmer Spectrum 100)	26
2.17	Sample in contact with evanescent wave	27
2.18	Light path of a phase contrast microscope	28
2.19	A Nikon Eclipse TS100 phase contrast microscope	29
2.20	Schematic diagram of an AFM	30
2.21	Atomic force microscopy XE-100 Park System	30
2.22	FE-SEM schematic diagram	32
2.23	Field Emission Scanning Electron Microscopy (JEOL JSM-7600F series)	32
3.1	The project flow chart	35
3.2	Flow chart of surface tension methods	36
3.3	Setup for contact angle measurement technique	37
3.4	Circuit diagram of contact angle motor controller	38
3.5	Flow chart to measure the contact angle of a droplet of fluid	39
3.6	Open pushbutton in MATLAB GUI	39
3.7	Command for OPEN pushbutton	40
3.8	Distilled water interacts with PDMS	40
3.9	Command for SNAP pushbutton	41
3.10	Command for SAVE pushbutton	41
3.11	Image processing command including Browse function	41
3.12	An enlarged image of the selected point of a Acetone on PDMS surface	42
3.13	Tools in MATLAB GUI	43
3.14	Command for search critical surface tension	43
3.15	Push button in GUI for Fox Zisman graph	44



3.16	Cholesteryl ester liquid crystal formulation	45
3.17	Mixture of cholesteryl ester liquid crystal	46
3.18	CELC in cholesteric phase at room temperature	47
3.19	Differential pressure sensor ASP1400 (SENSIRION)	47
3.20	A schematic diagram of ASP 1400 pressure sensor with RS 232	48
3.21	Experimental setup for measuring surface tension using bubble pressure method	49
3.22	Sample drop at the end of capillary tip	49
3.23	CELC sample in DSC pan	50
3.24	Q20 Differential scanning calorimeter	51
3.25	Experimental setup using OES	52
3.26	Ocean Optics HR4000 spectrophotometer	52
3.27	CELC substrate immersed in cell culture media	53
3.28	Sample placed on the sample holder for analysis	53
3.29	CELC sample were placed on top of ATR crystal	54
3.30	Medium for cell harvesting	55
3.31	HaCaT cells were sub-cultured and were plated in each petri dish	57
3.32	HaCaT were sub-culture without and with liquid crystal	57
3.33	Image of silicon cantilever from the AFM system over the HaCaT cell sample	58
3.34	Glass coverslips with gold sputter coater	58
4.1	The prototype models of a designed contact angle measurement system	61
4.2	A DC motor controller circuit connected to Arduino UNO	61



4.3	Enlarged image for angles value at the intersection point a droplet of Acetone with solid surface.	62
4.4	Enlarge image of contact angles using angle tool shows intersection point and contact angle	62
4.5	Eight probe liquids droplets on PDMS and Polyimide surface	63
4.6	Fluid surface tension of the eight probe liquid generated in excel for PDMS and Polyimide	64
4.7	CELC in cholesteric form	66
4.8	Image of sessile drops in contact with CELC coated glass slides and their contact angles	67
4.9	A Fox-Zisman plot for cholesteryl ester liquid crystal	67
4.10	Hardware consists of ASP1400 pressure sensor	68
4.11	CELC in 0.4 second, 0.8 second and 1 second before the bubble burst	69
4.12	Enlarged image of hemisphere	69
4.13	Samples reading of surface tension using bubble pressure	69
4.14	Image of pendant drop digitized drop and edge detection	71
4.15	DSC shows heat flow as a function of temperature of cholesteryl liquid crystals. No exothermic or endothermic activities were observed at 37 °C	72
4.16	Intensity versus wavelength for CELC	73
4.17	Percentage of transmission versus temperature	74



4.18	Image comparison using phase contrast and cross-polarizing optical microscopy.	75
4.19	A cross-polarizing micrograph of cholesteric based lyotropic liquid crystals shows the wide band streaks and focal conic textures.	75
4.20	The surface of CELC after immersion in cell culture media for 24 hours, 48 hours and 72 hours	76
4.21	XRD for CELC and lyotropic layer of CELC	77
4.22	The FTIR spectrum of cholesteryl ester liquid crystals	78
4.23	HaCaT cells morphology on glass substrate and for 24, 48 and 72 hours	79
4.24	AFM images for keratinocytes cultured on a plain glass and liquid crystal substrates	80
4.25	Image of HaCaT cell plated on the glass substrate and liquid crystal substrate after 24, 48 and 72 hours	82
4.26	Thickness of HaCaTs membrane cultured with and without the presence of liquid crystal as the substrate	83
4.27	Line profiles of cells cultured on plain glass and liquid crystal substrate	84
4.28	Human keratinocyte cell morphology cultured on glass on FE-SEM	85
4.29	Human keratinocyte cell morphology cultured on cholesteryl ester liquid crystal on FE-SEM	86



LIST OF SYMBOLS

SYMBOL	DESCRIPTION
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α	- Radius of curvature at apex drop
\AA	- Angstrom
B	- Drop shape factor
C	- Carbon
cm	- Centimetre
cm ⁻¹	- Wavenumber
°C	- Degree celcius
d	- Interplanar spacing
γ or σ	- Surface tension
$\Delta\rho$	- Difference between densities
Φ	- Coordinate
θ	- angle
F	- Free energy
H	- Hidrogen
I	- Intensity
l	- litre
g	- Gravity
N	- Nitrogen
%	- Percentage
O	- Oxygen
P or p	- Pressure
R	- Radius
T	- Transmission
T_g	- Glass Transition
T_m	- Melting points
λ	- Wavelength
m	- metre

mA	-	Mili Amp
ml	-	Mili litre
mg	-	Mili gram
mN/m	-	Mili Newton per metre
mM	-	Micro molar
kW	-	Kilo Watt
kV	-	Kilo Volt
μ	-	micro



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LIST OF ABBREVIATIONS

SYMBOL	DESCRIPTION
5CB	- Pentylcyanobiphenyl
8CB	- 4'-n-octyl-4-cyano-biphenyl
ATR	- Attenuated Total Reflectance
AFM	- Atomic Force Microscopy
CELC	- Cholesteryl Ester Liquid Crystal
CST	- Critical Surface Tension
DMEM	- Dulbecco's Modified Eagles's Medium
DMSO	- Dimethyl Sulfoxide
DNA	- Deoxyribonucleic Acid
DSC	- Differential Scanning Calorimeter
DSCG	- Disodium Cromoglycate
ECM	- Extracellular Matrix
EG	- Ethylene Glycol
FESEM	- Field Emission Scanning Electron Microscope
Fmoc	- Fluorenylmethyloxycarbonyl
FTIR	- Fourier Transform Infrared Spectroscopy
GND	- Ground
GUI	- Graphic User Interface
HaCaTs	- Human Keratinocytes
HBSS	- Hanks Balance Salt Solution
IPA	- Isopropyl alcohol
IR	- Infrared
LC	- Liquid Crystal
LCD	- Liquid Crystal Display
LCPA	- Liquid Crystal Pixel Array
LED	- Light Emitting Diode
LLC	- Lyotropic Liquid Crystal
MBBA	- 4-methoxybenzylidene-4'-n-butylaniline

MMA	-	Methylmethacrylate
MTT	-	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
OES	-	Optical Emission Spectroscopy
PEGA	-	Thermolysinpolyethylene Glycol Acrylamide
POM	-	Polarizing Optical Microscopy
PSPD	-	Position Sensitive Photo Diode
PWM	-	Pulse Width Modulation
SD	-	Standard deviation
TV	-	Television
UV	-	Ultraviolet
UTHM	-	Universiti Tun Hussein Onn Malaysia
V	-	Volt
Vis	-	Visible
XRD	-	X-ray Diffraction



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CHAPTER 1

INTRODUCTION

1.1 Project background

Liquid crystal (LC) is a new class of biomaterial that gained attention for usage in biomedical engineering. They provide means for label free observation of biological phenomenon such as non-toxic thermotropic liquid crystal overlaid with matrigel that had been used in recognizing restructuring of mammalian cells [1, 2]. Recently, a research found that [3], cholesteryl ester liquid crystals (CELC) containing cholesteryl moieties may have suitable biological affinity and also enable cell adhesion and proliferation without the need for pre-treatment with extracellular matrix molecules.

Due to the compact molecular arrangement, liquid crystal was shown to have high resolution on sensing ability in detecting localized cell exerted force and could be used for cell traction force sensor [4]. This is an important property that may reveal the potential of liquid crystal in a biomaterial to function on inert surface. Cell-compatible materials are very important in many biomedical applications [5-7]. Moreover, the cholesterol derivative in the liquid crystal was a well known mesogenic nature with their potential to self order into liquid crystalline substances.

However, the use of liquid crystal in biological study has not been fully explained. In 1857, Mettenheimer used a polarising light microscope and observed double refractility and black formed cross image composed of cholesterol from blood. Researchers proved that, occurrence of anisotropic myelin-like bodies in fatty streaks of the atheromatous lesions of the aorta of man [8]. The anisotropic basic structure has been interpreted as that of liquid crystal. In our living system, liquid crystalline order of molecule occurs in degenerating tissues of liver, spleen, lung and thyroid in the form of small spheres which appear under a conventional light

microscope as highly retractile and with double contours at their edges having the inner ring brighter than the outer ring [8].

This type of liquid crystal contains cholesteryl moieties which have affinity for cell membranes and have the abilities to change their properties. Cholesteryl moieties homeostasis is an important element in cellular membrane traffic and cell survival [9]. Therefore, this type of biomaterial is attractive as substrates because it could provide universally fundamental molecule to mammalian cells. Cholesteryl ester are considered to be easily biodegradable thus making the system suitable for the preparation of tissue engineering that sustain cell proliferation and migration [10]. The advantage of using liquid crystals as the biomaterial is it being non toxic and sensitive to biological interactions [11].

In addition, in order to study the biocompatibility of a biomaterial, surface energy property and thermal stability of a biomaterial are important factors in attracting the adhesion of cells and may affects cell surface interface [12]. Hence, finding surface tension of CELC was an important factor in this study. Moreover, biomaterials are majorly reliant on the surface energy [13] and stable over certain temperature. However, from the perspective of biophysical compability, little publication has been studied on the physical properties of liquid crystal. Through this study, synthesis and characterisation of surface properties CELC to interact with human keratinocytes (HaCaT) cells were reported. Furthermore, surface properties of CELC materials that promoted cell affinity were presented in this thesis.

1.2 Problem statement

In liquid crystal based biosensor development, an exposure to cell culture media is unavoidable and this may alter the surface properties of the liquid crystals over time. After immersion in cell culture media, the hydrophilic head and hydrophilic tails of the liquid crystal molecules would reoriented to interface with the water at the surface of the bulk liquid crystal as reported in [4]. However, the wettability of the surface or the surface tension of these liquid crystals in attracting the adhesion of cells have not been clearly identified and understood. Furthermore, the chemical properties of the CELC used in cell culture remain unknown. Although new applications of liquid crystals have been revealed [4], little is known about the

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